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Research Article

ANTI BACTERIAL POTENTIAL OF ETHANOLIC EXTRACT OF *Syzgium aromaticum* L. AND PHYTOCHEMICALS ISOLATION BY HPTLC

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Abstract

Organic extract of buds of *syzgium aromaticum* were screened for their antimicrobial activity against two humam pathogenic bacteria by disc diffusion assay. The pattern of inhibition varied with the various concentration of solution solvent used for extraction and the microorganism tested .among the ethanol extract, have potential to make inhibition zone against *E.coli and Staphylococcus aureus* . The results obtained with bud extract activity, a property that supports traditional use of their plants in treatment of some disease as broad spectrum antimicrobial agents. The presence of flavanoids was also identified by HPTLC.

Keywords: antimicrobial activity, *E.coli, staphylococcus aureus*, HPTLC.

INTRODUCTION

The science dealing with the study of the prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. Its sub disciplines are virology (study of viruses), bacteriology (study of bacteria), mycology (study of fungi), Phycology (study of algae) and Proto-zoology (study of protozoa). For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents.

These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defence mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi).

CLASSIFICATION OF ANTIBACTERIAL AGENTS

The antibacterial agents are classified in three categories:

Antibiotics and chemically synthesized

- Chemotherapeutic agents
- Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives)
- Immunological products.

(I) Antibiotics

They are produced by micro-organisms or they might be fully or partly prepared by chemical synthesis. They inhibit the growth of micro-organisms in minimal concentrations. Antibiotics may be of microbial origin or purely synthetic or semi synthetic. They can be classified by manner of biosynthesis or chemical structure. Structurally, they are classified into different classes as shown in the following table.

Mode of action

Antimicrobial drugs interfere chemically with the synthesis of function of vital components of micro organisms such as the cellular structure and functions of eukaryotic cells of the human body. These differences provide us with selective toxicity of chemotherapeutic agents against bacteria.

Antimicrobial drugs may either kill microorganisms outright or simply prevent their growth.

There are various ways in which these agents exhibit their antimicrobial activity. They may inhibit

- Cell-wall synthesis
- Protein synthesis
- Nucleic acid synthesis
- Enzymatic activity
- Folate metabolism or
- Damage cytoplasmic membrane

Table 1: Classification of antibiotics based on their chemical structure (Berdy, 1974)

S.No.	Name of the group	Examples
1.	**Carbohydrate-containing antibiotics**	
	Pure sugars	Nojirimycin
	Aminoglycosides	Streptomycin
	Orthosymycins	Everninomicin
	N-Glycosides	Streptothricin
	C-Glycosides	Vancomycin
	Glycolipids	Moenomycin
2.	**Macrocyclic lactones**	
	Macrolide antibiotics	Erythromycin
	Polyene antibiotics	Candicidin
	Ausamycins	Rifamycin
	Macrotetrolides	Tetranactin
3.	**Quinones and related antibiotics**	
	Tetracyclines	Tetracycline
	Anthracyclines	Adriamycin
	Naphthoquinones	Actinorhodin
	Benzoquinones	Mitomycin
4.	**Amino acid and peptide antibiotics**	
	Amino acid derivatives	Cycloserine
	β-Lactum antibiotics	Penicillin
	Peptide antibiotics	Bacteriacin
	Chromopeptides	Actinomycins
	Depsipeptides	Valinomycin
	Chelate forming peptides	Bleomycins
5.	**Heterocyclic antibiotics containing oxygen**	
	Polyether antibiotics	Monensin
6.	**Heterocyclic antibiotics containing Nitrogen Nucleoside antibiotics**	Polyoxins
7.	**Aromatic antibiotics**	
	Cycloalkane derivatives	Cycloheximide
	Steriod antibiotics	Fusidic acid
8.	**Aliphatic antibiotics**	
	Benzene derivatives	Chloramphenicol
	Condensed aromatic antibiotics	Griseofulvin
	Aromatic ether	Novobiocin

MEDICINAL PLANTS

Medicinal plants represent a rich source of antimicrobial agent. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.,* 1996) a wide range of medicine plants parts in used for extract as raw drugs and they posses varied medicinal properties. The different parts used include roots, stem, flowers fruits, twigs exudates and modified plant organ. While some of these raw drugs are collected in smaller quantities by the local communities and folk for local used, many other raw drugs are collected in larger quantities and treated in the market as the raw material are many herbal industries.Although hundreds of plant species have not been adequately evaluated (Balandrin *et al*., 1985). From the various investigations, in our present study the plant *Syzgium aromaticum* was chosen for antimicrobial activity.

MATERIALS AND METHODS

Collection of plant material

The fresh flowers of *Syzgium aromaticum* were purchased from Kandhsaras local market, Thanjavur.

Preparation of extract

The fresh flowers of *Syzgium aromaticum* were allowed to undergo extraction in Soxhlet apparatus for about 72 hours. The extract was allowed to undergo evaporation by means of kept in a boiling water bath to remove the alcohol. The alcohol free extract was used in the HPTLC analysis.

Phytochemical Screening

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods.

Antimicrobial activity

Disc diffusion method

Procedure

The culture media is inoculated when the medium maintains a temperature of 45°C that the cells can be distributed thoroughly ,then the medium containing the culture is poured in the sterilized petriplates, allow to solidify and the incubated colonies appear on the agar surface. Filter paper disc of 6mm diameter was punched with paper punching machine .The discs were then immersed with 1ml of the methanol. The disc were incubated for 24 hours and then dried at room temperature. The dried disc were sterilized by kept under incubator for an hour and then the plate was incubated at 37°C. The antibacterial activity was recorded by measuring the width of the clear zone around each colony.The zone of inhibition was measured to the nearest millimeter (mm).

Preparation of Antibiotic disc

Commercially available ampicillin antibiotic are used for this study. The above antibiotic was prepared for disc by dissolving antibiotic powder in 100ml of distilled water.

Minimal Inhibition Concentration (MIC)

MIC of the extract were also carried out using broth dilution method as described in (Ibekew *et al .,2001).* The nutrient broth and dextrose liquid were prepared according to the manufacturer's instruction (10ml of each broth was dispensed into separate test –tube and was sterilised at 121°c for 15 minutes and then allowed to cool. Two – fold serial dilution of the extracts in the broth were made from the stock concentration of the extract of 10, 5.2, 1.25, 0.625 mg/ml for water and ethanol extracts . 0.1 ml of the standardized inoculums of the microbes was inoculated into the different concentration of the extracts in the broth .The test tubes of the broth were incubated at 37°c for 24 hours and 30°c for 1-7 days for bacteria respectively and observed for turbidity. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

RESULTS AND DISCUSSION

The analysis of various concentrations of *Syzgium aromaticum* with numerous activities such as preliminary phytochemical analysis, antibacterial activity, HPTLC results were shown below.

Preliminary Phytochemical analysis

Table 1 depicts the result of preliminary phytochemical analysis of ethanolic extract of *Syzgium aromaticum* flowers. It shows the presence of Saponins, Tannins, Flavonoids, Terpenoids, Coumarin, Quinones, Lignins, Proteins and Phenols.

HPTLC

The HPTLC analysis of isolation of flavonoids are enclosed herewith

Antimicrobial activity

Table 2 depicts the antimicrobial potential of *Syzgium aromaticum* against *staphylococcus aureus* was briefly described along with their zone of inhibition.

Table 3 depicts the antimicrobial potential of *Syzgium aromaticum* against

Escherichia coli was briefly described along with their zone of inhibition.

Table 1: Preliminary Phytochemical Analysiss

S.No.	Name of the compound	Result
1.	Saponins	_
2.	Tannins	+
3.	Steroids	+
4.	Flavanoids	+
5.	Coumarins	_
6.	Alkaloids	+
7.	Phenols	+
8.	**Proteins**	_

+ = Presence, - = Absence

Table 2: Effect of *Syzgium aromaticum* against *staphylococcus aureus*

S.No	Name of the microorganism	Concentration (µg/ml)	Zone of inhibition (mm)
1.	*Staphylococcus aureus*	5	6.66 ±0.577
2.		10	11.66 ±0.577
3.		15	17.66 ±0.577
4.		20	21.83 ±0.721
5.		25	27.06 ±0.923
6.		30	38.86 ±0.750

Values are expressed as Mean± SD for 3 samples in each group; Students "t" test is followed ***P<0.05

Figure1: Effect of *Syzgium aromaticum* against *staphylococcus aureus*

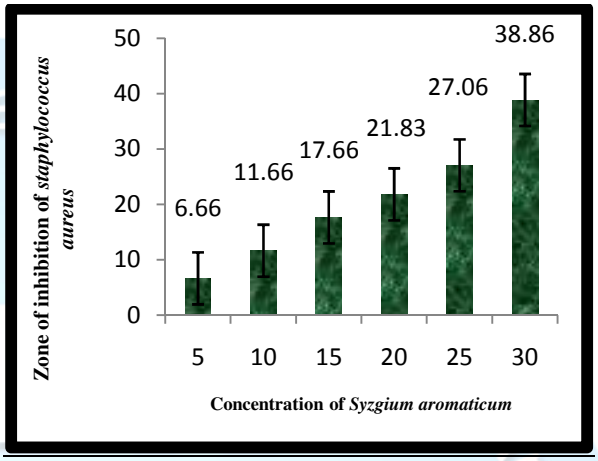
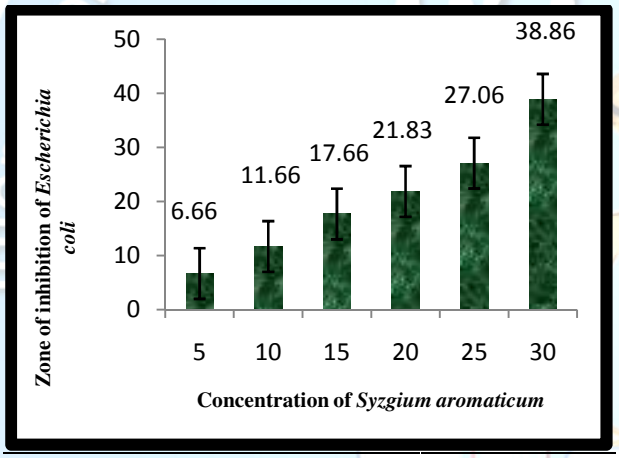
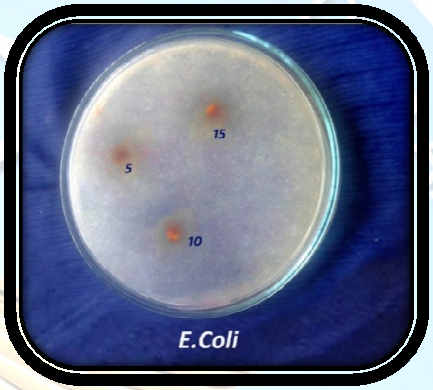


Plate 1: *Staphylococcus aureus* – Disc Diffusion Method

Figure 2: Effect of *Syzgium aromaticum* against *Escherichia coli*





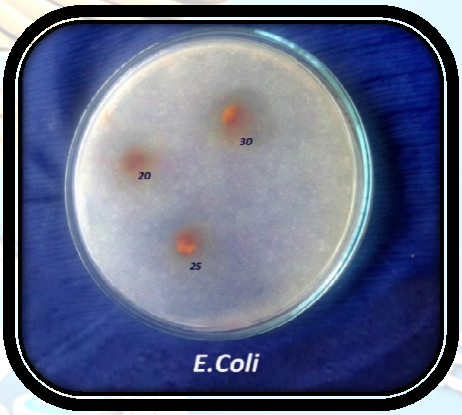


Plate 2: of *Escherichia coli* – Disc Diffusion Method

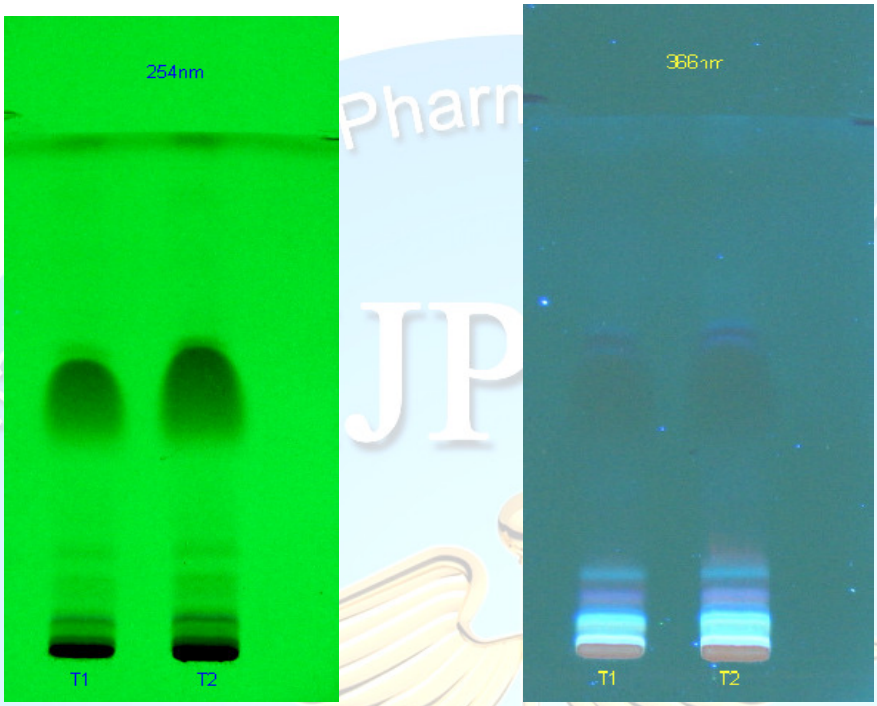
Table 3: Effect of *Syzgium aromaticum* against *Escherichia coli*

	Name of the microorganism	Concentration (µg)	Zone of inhibition (mm)
1.	*Escherichia coli*	5	9.66±0.5/ml77
2.		10	14.66±0.577
3.		15	18.66±0.577
4.		20	23±0.866
5.		25	26±0.866
6.		30	31.83±0.721

Values are expressed as Mean± SD for 3 samples in each group; Students "t" test is followed ***P<0.05

HPTLC Fingerprinting Profile for ethanolic extract of *Syzgium aromaticum* PHOTO DOCUMENTATION UNDER UV

AT 254nm **AT 366nm**



TLC DETAILS

T1&T2-10, 15µl of test solution

PEAK DISPLAY @ 254nm

PEAK DISPLAY (10µl of Sample)

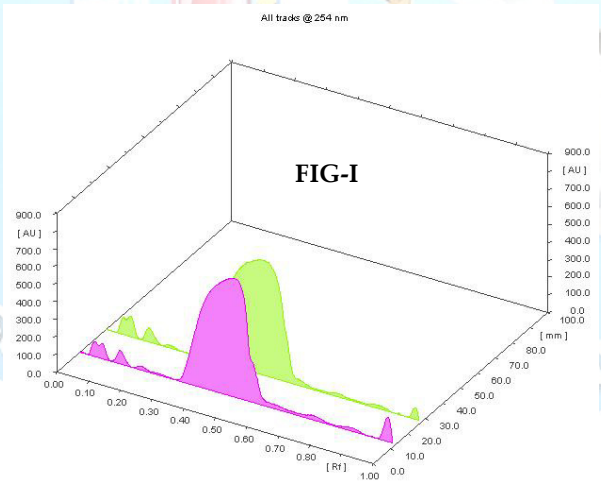
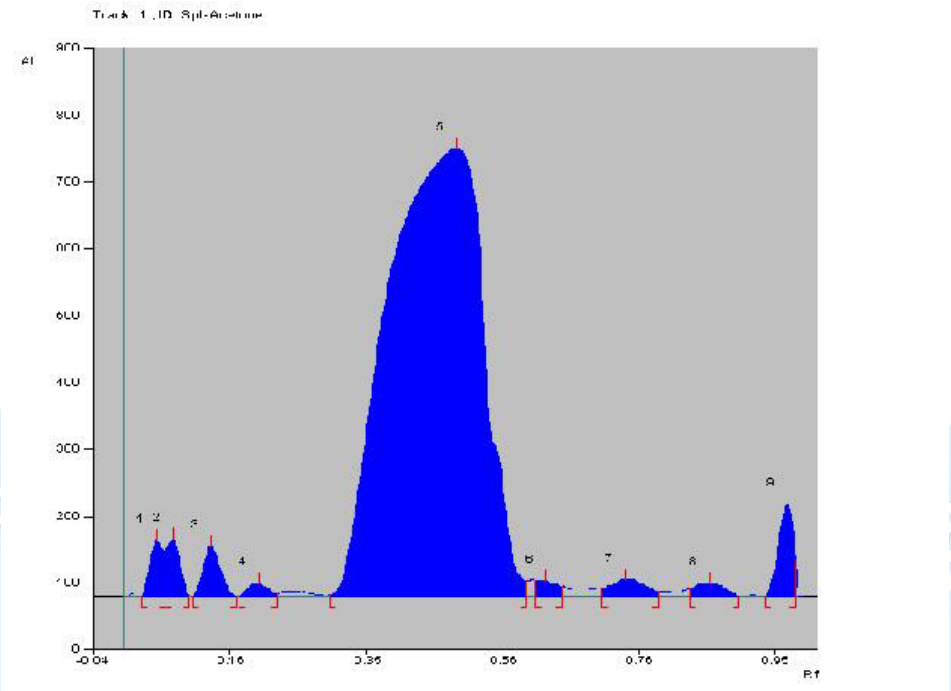
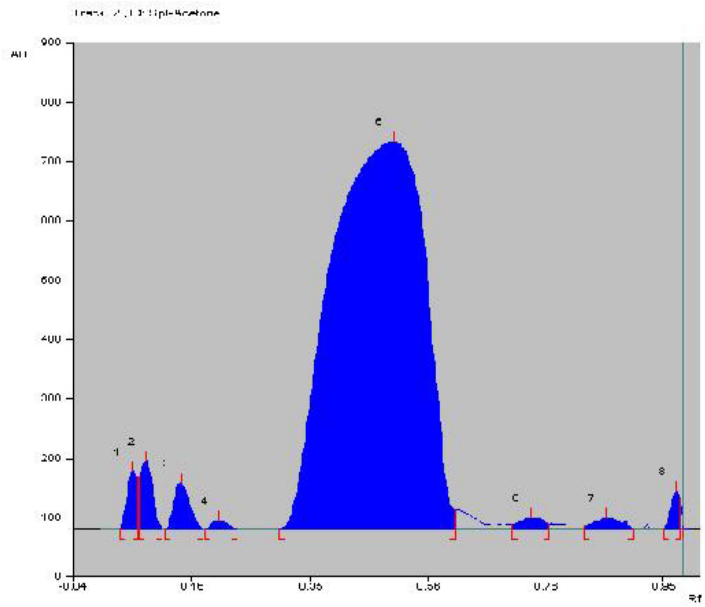


FIG-I



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.03	0.2	0.05	83.6	7.35	0.06	66.8	1213.8	1.50
2	0.06	67.8	0.07	84.8	7.44	0.10	0.5	1229.6	1.52
3	0.10	0.3	0.13	74.9	6.58	0.16	0.1	1403.4	1.74
4	0.17	0.3	0.20	19.8	1.74	0.22	4.0	424.4	0.53
5	0.30	1.7	0.49	669.9	58.84	0.59	22.2	72230.6	89.36
6	0.60	21.8	0.62	23.2	2.04	0.64	14.8	520.0	0.64
7	0.70	11.7	0.74	25.1	2.21	0.78	6.2	960.5	1.19
8	0.83	9.7	0.86	19.2	1.68	0.90	0.2	626.8	0.78
9	0.94	0.4	0.97	138.0	12.12	0.99	51.8	2218.6	2.74

FIG-II



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.03	0.7	0.05	97.8	9.19	0.06	87.7	1276.9	1.48
2	0.07	88.1	0.08	115.5	10.86	0.10	0.5	1861.2	2.15
3	0.11	0.5	0.14	79.2	7.44	0.17	0.3	1570.8	1.82
4	0.18	0.1	0.20	14.8	1.39	0.23	0.1	294.0	0.34
5	0.30	0.0	0.50	653.4	61.41	0.60	33.4	79533.7	91.94
6	0.70	8.0	0.73	19.5	1.83	0.76	9.0	609.8	0.70
7	0.82	6.2	0.86	19.0	1.78	0.90	2.2	677.7	0.78
8	0.96	0.1	0.98	64.9	6.10	0.98	53.1	684.6	0.79

HPTLC

HPTLC of hydro-alcoholic extract of drug on silica gel using Toluene. Ethyl acetate (9.9:0.1) as mobile phase shows eight spots at Rf value0. 05,0.08,0.14,0.20,0.50,0.73,0.86,0.98. This indicates the presence of flavonoids in our selected plant. The flavonoids has the nature of antimicrobial potential against pathogenic microorganism.

E. coli

In 1885, the German-Austrian pediatrician Theodor Escherich discovered this organism in the feces of healthy individuals. He called it *Bacterium coli commune* because it is found in the colon. Early classifications of prokaryotes placed these in a handful of genera based on their shape and motility (at that time Ernst Haeckel's classification of bacteria in the kingdom Monera was in place).(Farrar J, Hotez P,1967) Most *E. coli* strains do not cause disease but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia.

The main stay of treatment is the assessment of dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown

to shorten the course of illness and duration of excretion of ETEC in adults in endemic areas and in traveller's diarrhoea. The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are fluoroquinolones or azithromycin, with an emerging role for rifaximin. Oral rifaximin, a semisynthetic rifamycin derivative, is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveller's diarrhoea.

Staphylococcus aureus

Staphylococcus aureus is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as MRSA is a worldwide problem in clinical medicine. Spread of *S. aureus* (including MRSA) generally is through human-to-human contact, although recently some veterinarians have discovered the infection can be spread through pets, contamination thought to play a relatively unimportant part. Emphasis on basic hand washing techniques are therefore effective in preventing its transmission. The use of disposable aprons and gloves by staff reduces skin-to-skin contact and, therefore, further reduces the risk of transmission. Our plant with concentration of 30µg/dl exhibits potent antimicrobial activity, which is screened by zone of inhibition.

Summary and conclusion

Medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agent in the extract which was classified as active antimicrobial compounds .plant have the capacity to synthesize a diverse array of chemical and understanding how phytochemicals function in plant may further understanding of the mechanism by which they benefit humans. In plants, these compounds function and respond to environmental changes. In humans, they can have complementary and overlapping action, enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroids metabolism and antibacterial and antiviral effect.

The present study preliminary phytochemicals reveals the presence of flavanoids, alkaloids, steroids, phenols, tannins etc., From HPTLC analysis it cames to know that the presence of flavanoids with different Rf values were estimated.

In this study highest antimicrobial activity were noted in ethanolic extract of *syzgium aromaticum* highly inhibit the growth of *Eschericia coli* at 30µg/ml and for *staphylococcus aureus* the concentration of inhibition is 30µg/ml.

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